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Clinical evaluation of commercial SARS-CoV-2 serological assays in a malaria endemic setting

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ABSTRACT

The levels of immune response to SARS-CoV-2 infection or vaccination are poorly understood in African populations and is complicated by cross-reactivity to endemic pathogens as well as differences in host responsiveness. To begin to determine the best approach to minimize false positive antibody levels to SARS-CoV-2 in an African population, we evaluated three commercial assays, namely Bio-Rad Platelia SARS-CoV-2 Total Antibody (Platelia), Quanterix Simoa Semi-Quantitative SARS-CoV-2 IgG Antibody Test (anti-Spike), and the GenScript cPass™ SARS-CoV-2 Neutralization Antibody Detection Kit (cPass) using samples collected in Mali in West Africa prior to the emergence of SARS-CoV-2. A total of one hundred samples were assayed. The samples were categorized in two groups based on the presence or absence of clinical malaria. Overall, thirteen out of one hundred (13/100) samples were false positives with the Bio-Rad Platelia assay and one of the same one hundred (1/100) was a false positive with the anti-Spike IgG Quanterix assay. None of the samples tested with the GenScript cPass assay were positive. False positives were more common in the clinical malaria group, 10/50 (20%) vs. the non-malaria group 3/50 (6%); $p = 0.0374$ using the Bio-Rad Platelia assay. Association between false positive results and parasitemia by Bio-Rad remained evident, after adjusting for age and sex in multivariate analyses. In summary, the impact of clinical malaria on assay performance appears to depend on the assay and/or antigen being used. A careful evaluation of any given assay in the local context is a prerequisite for reliable serological assessment of anti-SARS-CoV-2 humoral immunity.

1. Introduction

Since the designation of SARS-CoV-2 as a global pandemic by the World Health Organization (WHO) in March 2020 (World Health Organisation (WHO), 2020), there has been an intense interest in developing reliable serological assays to measure humoral immune responses to SARS-CoV-2 following natural infections or immunization. Dozens of assays have received Emergency Use Authorization (EUA) by appropriate regulatory bodies such as the US Food and Drug Administration (Food and Drug Administration (FDA), 2022). However, assessment of the performance of the serological assays was mostly conducted in clinical samples collected from individuals living in high income countries, who are often not exposed to the same pathogens and live in different environments compared to people living in low- and middle-income countries. Specifically, very little is known about the performance of commercial SARS-CoV-2 assays in the context of Sub-Saharan Africa, where people are heavily exposed to various endemic pathogens and hence may exhibit pre-existing immune responses that

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cross-react with antibodies and/or antigens used to develop SARS-CoV-2 serological assays, thereby leading to false positivity and overestimation of exposure to SARS-CoV-2.

Earlier reports conducted in the African continent have revealed high anti- SARS-CoV-2 seroprevalence rates in the general population despite a limited number of laboratory-confirmed cases by molecular methods (Hajissa et al., 2022; Sagara et al., 2022; Uyoga et al., 2021). The discrepancy between seroprevalence data and the number of confirmed cases by molecular tests has raised concerns regarding the performance of the commercial test being used to detect SARS-CoV-2-specific antibodies in African samples (Nkuba Ndaye et al., 2021). Indeed, performance of serological assays can vary across populations, as evidenced by false positive Zika (Schwarz et al., 2017) and HIV (Gasasira et al., 2006) antibody responses in people exposed to *Plasmodium*, the parasite responsible for malaria, the most predominant infectious disease in Sub-Saharan Africa with an estimated 228 million cases in 2020 (World Health Organisation (WHO), 2021). Similar unsatisfactory performance during acute or past malaria episodes could be happening with SARS-CoV-2 serological assays as indicated by recent publications noting an increase in false positive responses in malaria-endemic African countries such as Nigeria, Ghana, Tanzania, and Zambia when compared to malaria naïve populations from the US (Emmerich et al., 2021; Tso et al., 2021a). The mechanism(s) driving such cross-reactivity is poorly understood and whether all types of commercial assays are impacted the same way is unclear.

Therefore, the present study was designed to evaluate the performance characteristics of three commercially available SARS-CoV-2 serology assays measuring antibody response against three widely used SARS-CoV-2 antigens, namely the Nucleocapsid (NCP), the Spike protein (S), and RBD domain of the Spike protein (RBD). We tested samples collected from individuals with and without clinical malaria before the COVID-19 pandemic in Mali, West Africa to determine any impact of clinical malaria, on the different SARS-COV-2 serological assays. Such testing should be a pre-requisite for selecting the appropriate assay(s) for measuring antibodies to SARS-CoV-2 in malaria endemic areas.

2. Materials and methods

2.1. Study population

A total of one hundred (100) plasma samples were collected between 2010 and 2018 from individuals living in Dangassa and Nioro in Mali, West Africa. Those individuals were enrolled as participants of clinical research protocols (N°09–39/FMPOS; N°2016/144/CE/FMPOS) approved by the institutional review board (IRB) of the University of Sciences, Techniques, and Technologies of Bamako in Mali. The samples were categorized in two groups. Group 1: Clinical Malaria ($N = 50$ samples) and Group 2: Healthy controls (N = 50 samples). Clinical malaria was defined by the presence of asexual forms of *Plasmodium falciparum* in blood smear by microscopy and a body temperature > 37.5 °C. Being healthy was defined as having a negative blood smear (i.e. no *Plasmodium* parasites detected by microscopy) and a normal body temperature (≤37.5 °C).

2.2. SARS-CoV-2 serology

Three commercial serological tests measuring anti-SARS-CoV-2 antibodies were used: Bio-Rad Platelia SARS-CoV-2 Total Antibody; Quanterix Simoa Semi-Quantitative SARS-CoV-2 IgG Antibody Test (anti-Spike); and the GenScript cPass™ SARS-CoV-2 Neutralization Antibody Detection Kit according to manufacturer instructions. These assays were selected given their ability to measure either total anti-SARS-CoV-2 antibodies (IgM/IgA/IgG) against SARS-CoV-2 nucleocapsid (NCP) [Bio-Rad], anti-Spike (S) IgG antibodies [Quanterix], or neutralizing antibodies to SARS-CoV-2 RBD domain of the Spike protein [GenScript]. All three assays had received Emergency Use Authorization by the FDA prior to initiating the study. Detailed characteristics of each assay are presented in Table 1. All samples were run in duplicate.

2.3. Data analysis

Data were exported in an excel spreadsheet and analyzed using GraphPad Prism and R studio version 4.2.2. We used the chi squared test to compare frequencies of seropositivity according to malaria status. In addition, we performed logistic regression to determine if the probability of having a positive test with the Bio-Rad assay depends on parasitemia after adjusting for age and sex. Likewise, a linear regression was run to assess if there is a relationship between the specimen ratio as a continuous variable and parasitemia, independently of age and sex. The specimen ratio of a given sample was calculated by dividing the mean OD value of the sample by the cut-off value. The later one was obtained by averaging the optical densities of the cut-off Control R4 of the Bio-Rad kit. Two-tailed p-values <0.05 were considered significant.

3. Results

3.1. Clinical characteristics of study participants

Pre-pandemic specimens were obtained from adult and pediatric subjects as indicated in Table 2. The median age of participants included in the study was 13 years with an age range between 1 and 72 years. The median age per study group was 15.5 years and 11 years, respectively for the healthy and malaria groups. Our study cohort was balanced in terms of sex with a sex ratio between males and females equal one, however the proportion between males and females in each group is different. As expected, the majority (76%) of individuals with clinical malaria had low hemoglobin levels (<12 g/dL) indicative of anemia, while only 20% of the healthy controls had such a low amount of hemoglobin. Among the group with clinical malaria, the median parasitemia was 13,925 trophozoites/μL. The parasitemia could be as low as 550 and as high as 38,125 trophozoites/μL, as indicated in Supplementary Table 2. Additionally, the majority of those with clinical malaria (64%) had a parasitemia higher than 10,000 trophozoites/μL. When we categorized our participants by clinical grade (mild to severe malaria), 10% of our cohort had severe malaria as shown in Table 2.

3.2. Seropositivity rate by assay and malaria status

To compare the performance of three commercial SARS-CoV-2 serological assays, all samples were run in duplicate for each assay. Among the 100 samples tested, 13 were positive for total antibodies with Bio-Rad and only 1 positive was found with Quanterix anti-Spike IgG assay (Fig. 1A). This gave an overall global seropositivity rate of 14% in pre-pandemic samples from Mali. The only sample which was positive (1283 ng/mL) with Quanterix was not detected by Bio-Rad. It should be noted that none of the samples had detectable neutralizing antibodies by GenScript surrogate neutralization assay (Fig. 1A). We then analyzed the data obtained by study groups (Clinical Malaria versus Healthy). Out of the 13 samples tested positive by Bio-Rad, 10 had malaria (Fig. 1B). The same was true for the only sample identified as positive with Quanterix (Fig. 1B). Among the clinical malaria group, 10 out of 50 samples tested (20%) were seropositive by Bio-Rad, as presented in Fig. 2. Meanwhile, among the healthy controls, 3 out of 50 samples (6%) were positive; $p = 0.0374$ (Fig. 2).

3.3. Relationship between seropositivity and parasitemia

Given that naturally acquired immunity to malaria is thought to be associated with age and the frequency of exposure to infectious mosquito bites and the possibility of sex-specific differences in immune responses to pathogens, we sought to rule out any effect of age and sex on

Table 1
Commercial serological SARS-CoV-2 assays used in the study.

Name	Platelia SARS-CoV-2 Total Ab	Simoa™ Semi-Quantitative SARS-CoV-2 IgG Antibody Test	cPass™ SARS-CoV-2 Neutralization Antibody Detection Kit
Manufacturer	Bio-Rad Laboratories Inc.	Quanterix Corporation	GenScript Inc.
Test Principle	Qualitative Antigen Capture ELISA Assay	Semi-Quantitative Paramagnetic Microbead-based Sandwich ELISA Assay	Qualitative direct ELISA assay
Test Format	96-well Plate	96-well Plate; tubes	96-well Plate
Antigen Targeted	Nucleocapsid	Spike	RBD domain of Spike
Antibody Isotype	IgA/IgM/IgG	IgG	Total neutralizing antibodies
Sample Dilution	1:5	1:1000	1:10
Instrument	Spectrophotometer (450/620 nm)	Simoa HD-X Analyser	Spectrophotometer (450 nm)
Read out	Optical Density	Fluorescence	Optical Density
Data Interpretation	Negative <0.8 Equivocal 0.8 ≤ x < 1 Positive ≥1	Negative <0.77 μg/mL Positive ≥0.77 μg/mL	Negative <30% Signal Inhibition Positive >30% Signal Inhibition
Sensitivity*	98.0% 95% CI (89.5%; 99.6%)**	100% 95% CI (87.9%; 100%)	100% 95% CI (87.1%; 100%)
Specificity*	99.3% 95% CI (98.3%; 99.7%)	99.2% 95% CI (97.9%; 99.7%)	100% 95% CI (95.8%; 100%)
Clinical Performance (comparison with gold standard)†	Positive percent Agreement: 92.16% Negative percent Agreement: 99.56%	Positive percent Agreement: 87.5%‡ Negative percent Agreement: 99.19%	Positive percent Agreement: 100% Negative percent Agreement: 100%

* Sensitivity and specificity data was obtained from the Food and Drug Administration (FDA) website updated on 22AUG2022 (https://www.fda.gov/medical-devices/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/eua-authorized-serology-test-performance).
** CI means Confidence Interval.
† Gold standard is RT-PCR for Bio-Rad and Quanterix assays, but Plaque Reduction Neutralization Test (PRNT) for GenScript.
‡ Data obtained when bleeding occurs between 8 and 17 days from RT-PCR positive test.

Table 2
Clinical characteristics of the pre-pandemic samples tested.

Characteristics	Healthy Control	Clinical Malaria	Total
	# (%)	# (%)	#
Age			
<18-year-old	28 (56)	38 (76)	66
>18-year-old	22 (44)	12 (24)	34
Sex			
Male	20 (40)	30 (60)	50
Female	30 (60)	20 (40)	50
Hemoglobin Level			
<12 g/dL	10 (20)	38 (76)	48
≥ 12 g/dL	40 (80)	12 (24)	52
Parasitemia			
<1000	–	2 (4)	2
1000-10,000	–	16 (32)	16
>10,000	–	32 (64)	32
Clinical grade			
Mild Malaria	–	45 (90)	45
Severe Malaria	–	5 (10)	5

the association observed between malaria status and false positivity as presented in Fig. 2. We run both logistics and linear regressions using data obtained with Bio-Rad on all samples. We did this analysis with Bio-Rad only since it appears to have the highest false-positivity rates (13%). After adjusting for age and sex, the increase of parasitemia was associated with the odd of having false positive test (aOR = 1.07; $p = 0.017$). A similar result was obtained when we assessed the association between parasitemia and the specimen ratio after controlling for any effects of age and sex (aCoeff: 0.026, $p = 0.00973$), suggesting that the likelihood of having a false positive test with Bio-Rad depends on parasitemia, independently of age and sex.

To visualize the relationship between positivity and parasitemia, we computed a figure displaying the specimen ratio obtained from all samples with Bio-Rad in relation with parasitemia levels (Fig. 3A) or age groups (Fig. 3B). Consistent with our regression analyses, the proportion of false positive results appeared to increase as function of parasitemia for up to 30,000 trophozoites/μL (Fig. 3A). In contrast, there does not appear to be an increase in the false positivity rate as age increases (Fig. 3B), as demonstrated in multivariate analyses earlier (Table 3). Specifically, similar proportions of false positive results were found between different age categories, indicating that age is not a major contributing factor for the increase of false positive results with the Bio-Rad assay.

4. Discussion

The need for having accurate SARS-CoV-2 serological assays in settings where other pathogens are endemic is increasingly being recognized. Here, we evaluated three commercially available SARS-CoV-2 serological assays in clinical samples collected from Mali prior to the COVID-19 pandemic. Selected assays, namely Bio-Rad Platelia SARS-CoV-2 Total Antibody, Quanterix Simoa Semi-Quantitative SARS-CoV-2 IgG Antibody Test, and the GenScript cPass™ SARS-CoV-2 Neutralization Antibody Detection Kit (cPass), have already received Emergency Use Authorization by the FDA to assess antibody responses to NCP, Spike protein, and RBD domain of SARS-CoV-2, respectively. The assays were conducted on well-characterized pre-pandemic plasma samples collected from individuals with or without clinical malaria. The proportion of false positive samples was 13% with BioRad and 1% with Quanterix. None of the positive samples were also found to have detectable neutralizing antibodies, supporting the notion that these were false positives. The Bio-Rad assay was the least specific in samples from patients with clinical malaria. An association was found between clinical malaria and false positivity using the Bio-Rad assay with 20% of individuals with clinical malaria being seropositive compared to just 6% of healthy controls. This relationship between false positivity and malaria was also confirmed in multivariate analyses after controlling for age and sex.

There is a growing body of literature describing seropositivity for SARS-CoV-2 in samples collected prior to COVID-19. A limited number of those have been conducted in Sub-Saharan Africa, where endemic pathogens including *Plasmodium*, Dengue virus, and previous human coronaviruses circulate. A study conducted by Tso and colleagues has revealed that cross-reactive responses to human coronaviruses were eight times higher in Tanzania and Zambia than the US (Tso et al., 2021a). In Mali, high background reactivity with no detectable

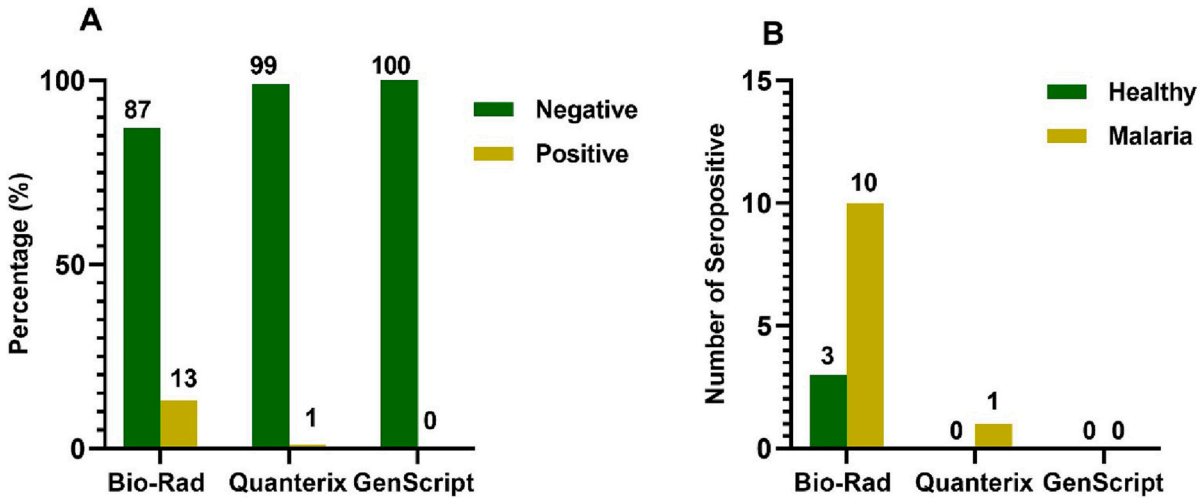

Fig. 1. Seropositivity rate in pre-pandemic samples by three different commercial serological assays and by malaria status.
Archived pre-COVID-19 plasma samples ($N = 100$) were tested using three commercial assays, namely Bio-Rad Platelia SARS-CoV-2 Total Antibody, Quanterix Simoa Semi-Quantitative SARS-CoV-2 IgG Antibody Test (anti-Spike), and the GenScript cPass™ SARS-CoV-2 Neutralization Antibody Detection Kit, as shown on the X-axis of each panel. Green and yellow bars in Fig. 1A represent percentage of negative and positive samples, respectively per assay type. Similarly, numbers on the top of the bar graphs indicate the percentage of positive and negative samples identified by each assay. In Fig. 1B, seropositivity was disaggregated based on the assay being used (on X-axis) and presence or absence of clinical malaria. Bar graphs show number of seropositive samples (on Y-axis) in malaria cases (yellow) and healthy individuals (green). Data labels on top of each graph represent number of seropositive samples as indicated on the Y-axis. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

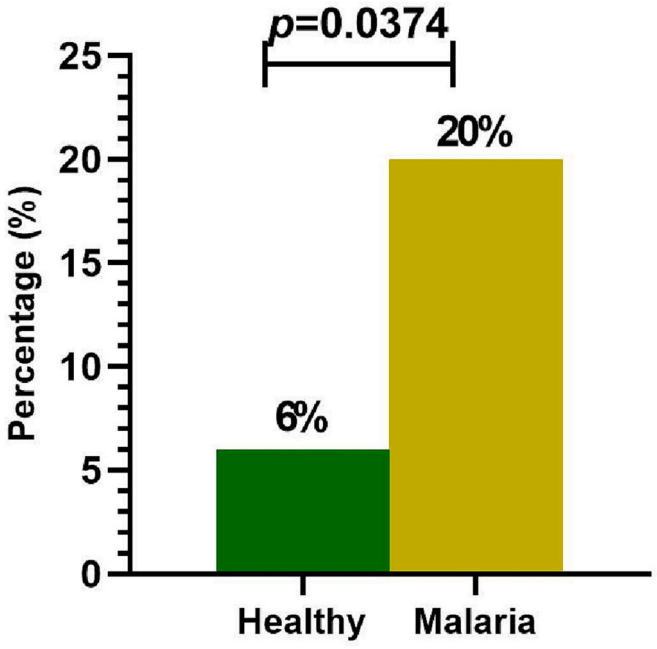

Fig. 2. Relationship between seropositivity and malaria status by Bio-Rad Platelia in pre-pandemic samples.
In this Figure, samples tested with the Bio-Rad Platelia assay were stratified based on malaria status, namely 50 Healthy (green bar) and 50 Malaria cases (yellow bar), as presented on the X-axis. The Y-axis shows the proportion of seropositive samples in each group, which is also indicated on top of each bar graph. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

functional activity was first reported by analyzing pre-pandemic samples using an in-house ELISA method (Woodford et al., 2021). Cross-reactive antibody responses in pre-COVID-19 samples was recently confirmed by another group from Mali (Traore et al., 2022). Such non-specific reactivity correlated with antibody responses to human coronaviruses responsible for the common cold (OC3, and HKU1), but not for antibodies targeting *Plasmodium* antigens (Woodford et al., 2021). Similarly in Benin, the difference between the proportion of false positives individuals with and without clinical malaria was not statistically significant (71.4% versus 54.3%; $p = 0,35$), albeit seropositive samples also had substantially elevated parasitemia (Yadouleton et al., 2021).

In the present study, we have not tested our samples for presence or absence of antibodies against common cold coronaviruses, however, we did find a significantly higher proportion of seropositivity in subjects with clinical malaria when compared to healthy controls using Bio-Rad assay. Our data are consistent with investigations conducted in Nigeria (Steinhardt et al., 2021), Senegal (Yansouni et al., 2022), and Cambodia (Manning et al., 2022). In those independent studies, a reduced specificity of SARS-CoV-2 serological assays was associated with malaria and/or presence of antibodies to malaria antigens. False positivity was substantially reduced upon urea wash or by using country-specific cut-off values (Steinhardt et al., 2021; Woodford et al., 2021). The reason for this difference between our study and previous reports in Mali could be due to the type of assay platforms being used (bead versus plate-based assays) or the antigen being targeted. This is because no false positivity was detected in *Plasmodium*-infected samples using the GenScript and only a low level with the Quanterix assay. Nevertheless, the performance observed in this study in term of specificity, namely 100% for GenScript and 99% for Quanterix, is very similar to that reported by FDA, as presented in Table 1.

The mechanism driving cross-reaction between SARS-CoV-2 and *Plasmodium* humoral immune responses has not been elucidated. A possible explanation could be non-specific activation of B cells during clinical malaria, which is known to induce production of polyclonal antibodies that are not pathogen-specific, thereby leading to false positive reactions (Scholzen and Sauerwein, 2013). A longstanding example has been noted with commercial serological assays to detect Zika virus infection in patients with past or present malaria (Van Esbroeck et al., 2016). However, this phenomenon is not restricted to *Plasmodium* infection since HIV infection or reactivation of herpes viruses such as CMV and EBV may also lead to polyclonal B cell activation. This has been noted in a recent study in Gabon during which 25% of pre-COVID-

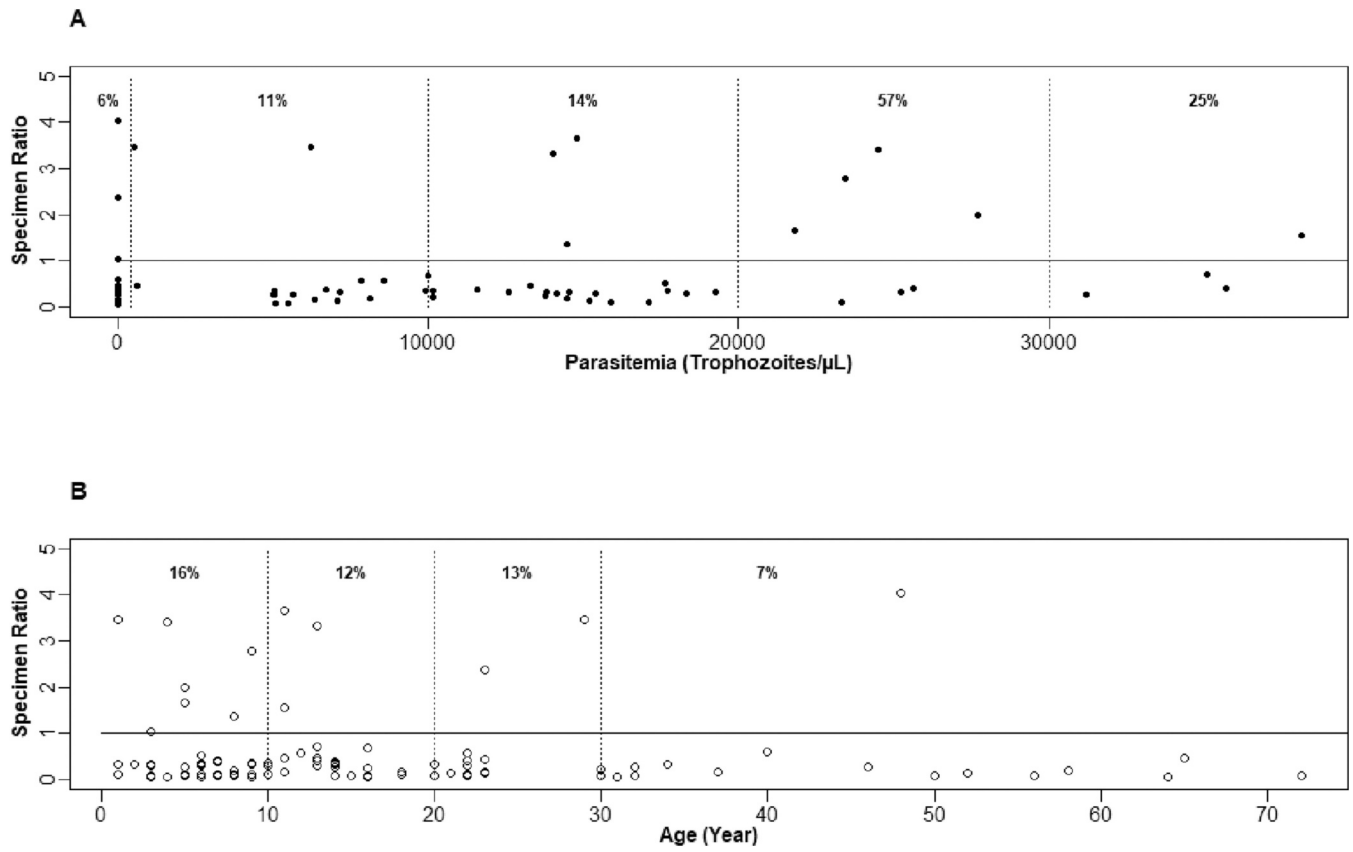

Fig. 3. Relationship between specimen ratio by Bio-Rad and parasitemia or age.
Each dot indicates a given sample analyzed with Bio-Rad. We plotted parasitemia (Fig. 3A) or age (Fig. 3B) on the X-axis against the specimen ratio (mean OD value of the sample/ cut-off value) on the Y-axis. Dotted vertical lines indicate parasitemia or age categories. Values in percentage in each category represent the proportion of false positive samples detected within each parasitemia or age category.

Table 3
Multivariate analyses among all participants by Bio-Rad.

Analytical approach	Logistic regression		Linear regression	
Variables	Seropositivity aOR [95% CI]	*p*-Value	Specimen ratio aCoeff. [95% CI]	*p*-Value
Age (year)	0.99 [0.94 to 1.04]	0.663	0.0017 [−0.0096 to 0.013]	0.714
Sex	1.30 [0.36 to 4.65]	0.690	−0.085 [−0.44 to 0.27]	0.639
Parasitemia (Trophozoites/ 1000 µL)	1.07 [1.01 to 1.13]	0.017	0.026 [0.0077 to 0.044]	0.00973

19 samples which tested positive by serology have detectable IgG responses to CMV (Mveang Nzoghe et al., 2021). On the other hand, an investigation led by Bei and colleagues, that is currently under review at the time of writing this report, suggests that cross-reactive antibodies during acute malaria can bind to terminal sialic acids residues of complex glycans of the Spike protein (Lapidus et al., 2021). Moreover, in our study those cross-reactive antibodies were not neutralizing, suggesting again a possible overestimation of humoral responses to SARS-CoV-2 in regions where malaria is endemic.

Despite the focus on malaria in this report, we cannot ignore the possibility that pre-existing humoral immunity to previous coronaviruses are also implicated. For instance, cross-reactivity of SARS-CoV-2 antigens with antibodies against the NCP of other human coronaviruses, including alphacoronaviruses (HCoV-NL63 and HCoV-229E) has been reported (Tso et al., 2021a). However, common cold coronaviruses also circulate in northern regions of the world (US and Europe), where false positivity in pre-pandemic samples was not evident, suggesting that cross-reactivity between SARS-CoV-2 and previous coronaviruses cannot fully explained our findings. Another possibility is cross-reactivity between anti-SARS-CoV-2 antibodies and humoral immunity to the Dengue virus, as shown by independent reports conducted in Indonesia and Singapore (Masyeni et al., 2021; Yan et al., 2020). We did not address this possibility in the current study because *Plasmodium* infection is more common in Mali compared to Dengue virus infection.

It should be noted that false seropositive results in this study were detected in assays primarily targeting NCP (13 out of 100 by Bio-Rad). Independent research groups have proposed that commercial assays utilizing recombinant NCP antigen are more sensitive but prone to false positive results when samples are originating from Sub-Saharan Africa (Emmerich et al., 2021; Tso et al., 2021a; Yansouni et al., 2022). This observation could explain the increased false positive rate with Bio-Rad when compared to Quanterix and GenScript assays in this study. Conversely, our data indicate that serological assays detecting anti-S or RBD domain of the S protein appeared to have higher specificity. As in our study, when GenScript was compared to two commercial serological assays detecting antibody responses to NCP, GenScript had a higher specificity in testing pre-pandemic samples collected from subjects infected with protozoans parasites, including *Plasmodium* (Yansouni et al., 2022). Together, there seems to be a tradeoff between sensitivity and specificity in term of commercial SARS-CoV-2 serological tests in an African context. Notably, detecting antibodies against S antigens seems more suitable for serological assays because of their relatively higher

specificity, perhaps resulting from genetic variability of the *S* gene. Conversely, assays detecting antibodies against NCP antigens appear to be more sensitive, but less specific and may be useful during an initial screening which has to be confirmed with an S-based serological assay to ensure a correct data interpretation.

Our study has several strengths, including 1) the use of a well characterized cohort of samples collected before emergence of SARS-CoV-2, in which presence or absence of clinical malaria has been determined, 2) evaluation of three different commercial assays that have all received Emergency Use Authorization by the US FDA, and 3) assessment of functional capacity of antibody responses. However, we also noted some limitations that need to be acknowledged. We have not tested our samples for cross-reactivity with common human coronaviruses, as well as other endemic viral, bacterial, and parasitic diseases. Also, we were not able to ascertain the direct impact of prior *Plasmodium* infections, specifically in the healthy control group, since antibody levels against *Plasmodium* were not determined. Having those pieces of data could have yielded a global picture of potential driving factors altering assay performance in addition to the presence or absence of clinical malaria. In addition, the commercial assays compared are not based on the same principle, nor focused on the same antigens or antibodies isotypes, thus some of the differences observed could be due to inherent differences between varying assay platforms or antigen/antibodies targeted. Finally, we have assessed antibody functionality by focusing on the capacity to inhibit the interaction between viral RBD and host cell receptor ACE-2 (Angiotensin-Converting Enzym-2) in vitro, but we have not assessed the ability of the antibodies to bind and activate immune cells via their cognate Fc receptor (i.e., antibody-dependent cytotoxicity), hence contributing to anti-viral activities as reported earlier (Tso et al., 2021b; Yu et al., 2021). Thus, we may not be able to completely ascertain an absence of functional antibody response in the pre-pandemic samples by solely focusing on inhibition of viral entry into host cells.

In conclusion, our data indicate that infection with *Plasmodium*, the parasite responsible for malaria, might be altering the performance of commercial serological assays that were validated in non-malaria endemic settings. Thus, any serologic assay for SARS-CoV-2 needs to be validated in the local region in which it is being used before its large-scale use in seroprevalence studies and clinical trials.

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Author contribution statement

DD, KSS and RD designed the study, analyzed and interpreted the results, and wrote the manuscript; DK, SAD, BK, BB, SS, MT, SD, MD enrolled and followed the participants for the study, developed clinical database, and reviewed the manuscript; HH, PL, TR, DD, DK, SAD, BK, MW, and NC processed the samples, collected the data, analyzed the data, and reviewed the manuscript; IS and SL offered new analytical approach for data analysis and interpretation and reviewed the manuscript; KSS, EA, HL, and RD coordinated the supply of new reagents, interpreted the results, critically reviewed, and edited the manuscript. All authors have approved the final version of the manuscript prior submission.

Ethical approval

Archived samples collected during clinical research protocols, which have already received IRB approval (No09–39/FMPOS; N°2016/144/CE/FMPOS) were used. In those protocols, participants gave consent to use their clinical information and samples for future research. Therefore, an additional ethical approval was not needed for the purpose of this study.

Declaration of Competing Interest

The authors declare no competing financial interests.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jim.2023.113488.

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